Acta Solum 4, Number 2 (March 2026), 100–109. DOI: 10.20527/actasolum.v4i2.3560
http://jtam.ulm.ac.id/index.php/actasolum e-ISSN: 2987-5145

Second-Year Biochar Residue and NPK Fertilizer Effects on Soil Mesofauna and Microbial Activity in Patchouli (*Pogostemon cablin* Benth.)

Any Kusumastuti, Made Same, Nurhasanah, AH. Maftuh Haifidh Zuhdi*, Dian Latifathul Mar'ah

State Polytechnic of Lampung, Bandar Lampung, Lampung 35141, Indonesia

*Correspondence author email: hafidhzuhdi@polinela.ac.id

Article Information

Received November 05th 2025
Accepted March 05th 2025
Published March 25th 2026
Online March 25th 2026

Keywords:
Biochar; Mesofauna; Microbial respiration; NPK application; Patchouli cultivation

Abstract

Second-year biochar residue represents an understudied aspect of long-term soil amendment management, as its persistence in soil may influence biological communities differently over time. This study aimed to analyze the effects of second-year biochar residue and NPK fertilizer rates on soil mesofauna abundance and soil microbial activity in patchouli (*Pogostemon cablin* Benth.) cultivation. The experiment was arranged in a split-plot design with three replications. The main plot factor was second-year biochar residue rates (0, 10, and 20 ton ha^{-1}), and the subplot factor was NPK fertilizer rates (0, 350, and 700 kg ha^{-1}). Second-year biochar residue had no significant effect on soil mesofauna population, diversity index, dominance index, or microbial activity, while NPK fertilizer at 700 kg ha^{-1} significantly increased mesofauna population (24.33 individuals dm^{-3}), diversity index (0.28), and dominance index (0.022). A total of 501 mesofauna individuals were identified, dominated by Acarina (67.3%) and Collembola (28.9%). Correlation analysis revealed strong positive relationships between mesofauna variables and soil physical factors, particularly temperature (r = 0.74) and moisture (r = 0.83), while soil chemical properties showed weak correlations. No significant interactions were observed between biochar residue and NPK fertilizer. These findings indicate that biochar's stimulatory effects on soil biota diminish substantially after the first year. In contrast, NPK fertilization at 700 kg ha^{-1} effectively enhances soil mesofauna communities, which serve as indicators of soil biological health in patchouli cultivation systems.

1. Introduction

Patchouli (*Pogostemon cablin* Benth.) is an essential-oil-producing crop of high economic value in the global perfume and cosmetics industry. Indonesia, as one of the world's major patchouli oil producers, faces challenges in productivity and the sustainability of cultivation. Data show a decline in the patchouli cultivation area from 18.63 thousand hectares in 2015 to 16.78 thousand hectares in 2024, with an average annual decline of 1.52% (Kementerian Pertanian, 2024). This decline in cultivated area is attributed to soil fertility degradation resulting from intensive cultivation practices without adequate soil amelioration. Continuous patchouli cropping has been reported to decrease soil pH and total potassium, thereby compromising soil health (Wang et al., 2024). Nisa et al. (2024) reported that patchouli monoculture produced the lowest Soil Quality Index compared with mixed-garden and intercropping systems, indicating that continuous single-crop cultivation degrades soil biological conditions over time. Degradation directly impacts soil biota, particularly mesofauna, which serve as bioindicators of soil health. Soil mesofauna, including Collembola, Acarina, and other microarthropod groups, perform essential ecological functions in the decomposition of organic matter, nutrient cycling, and the formation of soil structure (Menta and Remelli, 2020).

Rice husk biochar, produced by pyrolysis at 300–600°C under limited oxygen, has been shown to enhance soil quality by improving physical, chemical, and biological properties (Lehmann et al., 2021). In the first year of application on patchouli plantations, rice husk biochar at 20 ton ha^{-1} significantly increased soil mesofauna abundance and microbial activity (Kusumastuti et al., 2022). Biochar application increases cation exchange capacity, water and nutrient retention, and soil pH (Blanco-Canqui, 2017). However, the residual effects of biochar in the second year after application are a crucial aspect that requires in-depth investigation, given biochar's high persistence in soil, with half-lives ranging from hundreds to thousands of years (Wang et al., 2016). After one year of application, biochar undergoes stabilization and integration into the soil matrix, which can alter its impact on soil biota communities. Research on the impact of second-year biochar residue on soil biota communities, particularly mesofauna, remains very limited.

The combination of biochar with inorganic fertilizers such as NPK can provide synergistic effects in enhancing nutrient availability and soil biological activity (Agegnehu et al., 2016). Nitrogen, phosphorus, and potassium are essential macronutrients that play roles in plant growth and indirectly influence soil biota communities by increasing root biomass and root exudates, which serve as energy sources (Xiao et al., 2018). NPK fertilizer rates of 350–700 kg ha^{-1} applied in this study were determined based on agronomic recommendations from previous research on patchouli cultivation (Kusumastuti et al., 2022). However, inappropriate NPK fertilization can cause imbalances in microbial and mesofauna communities (Zhu et al., 2020). Soil microbial activity is an important indicator of soil ecosystem health and function, reflecting organic matter decomposition processes and nutrient mineralization (Trivedi et al., 2016). The interaction between second-year biochar residue at rates of 0, 10, and 20 ton ha^{-1} and NPK fertilization at rates of 0, 350, and 700 kg ha^{-1} can affect microbial activity by altering the availability of labile carbon and soil microenvironmental conditions (Sun et al., 2019).

Understanding of second-year biochar residual effects, particularly when combined with NPK fertilization, on mesofauna and soil microbial activity in patchouli cultivation remains very limited. This information is essential for developing sustainable cultivation practices that require an understanding of the long-term impacts of agronomic inputs on soil health. The integration of biochar residue effects with fertilization management needs to be addressed to optimize soil biological function in perennial crop systems. Therefore, this study aimed to analyze the effects of second-year rice husk biochar residue rates (0, 10, and 20 ton ha^{-1}) and NPK fertilizer rates (0, 350, and 700 kg ha^{-1}) on soil mesofauna abundance and soil microbial activity in patchouli (*Pogostemon cablin* Benth.) cultivation, and to identify optimal rates that can enhance soil biological health as an indicator of cultivation system sustainability.

2. Research Methods

2.1 Research Location

The study was conducted at the experimental field of the State Polytechnic of Lampung, Bandar Lampung, Indonesia, from July to September 2025. The experimental site is characterized by Ultisols ordo with an average annual rainfall of 1,500 mm. Soil mesofauna abundance and soil microbial activity analyses were performed at the Soil Science Laboratory of the State Polytechnic of Lampung.

2.2. Experimental Design

The experiment was arranged in a split-plot design with three replications. The main plot factor was the second-year biochar residue rate at three levels: without biochar residue (B0), biochar residue at 10 ton ha^{-1} (4.2 kg per 4 m^2 plot; B1), and biochar residue at 20 ton ha^{-1} (8.4 kg per 4 m^2 plot; B2). The subplot factor was the NPK compound fertilizer (16:16:16) rate at three levels: without NPK fertilizer (P0), NPK fertilizer at 350 kg ha^{-1} or 17.1 g $plant^{-1}$ (P1), and NPK fertilizer at 700 kg ha^{-1} or 34.3 g $plant^{-1}$ (P2). The biochar and NPK dosages were determined based on agronomic recommendations from previous research conducted on the same patchouli plantation (Kusumastuti et al., 2022). This resulted in nine treatment combinations with 27 experimental units. Each experimental unit consisted of nine patchouli plants (*Pogostemon cablin* Benth.) planted at 0.70 m × 0.70 m spacing in 4 m^2 plots.

2.3. Field Implementation

Rice husk biochar used in this study was the residue from the first-year application, integrated into the soil matrix. NPK fertilizer was applied in two split applications at one and two months after planting, at the treatment rates. NPK fertilizer was applied evenly by broadcasting around the plant base within the plot. Plant maintenance included irrigation once per day, weed control every two weeks, and pest and disease management as needed.

2.4. Sampling and Analysis

2.4.1. Soil Sampling

Soil samples were collected at 3 months after planting. Composite soil samples were formed from three sampling points per experimental unit using ring samples (100 cm^3) at 0-10 cm depth. Samples were placed in labelled

plastic bags and immediately transported to the laboratory for analyses of soil mesofauna abundance and microbial activity.

2.4.2. Soil Mesofauna Abundance Analysis

Soil mesofauna extraction was performed using the Berlese-Tullgren method (Krebs, 1989) with three replications per sample. 100 g of soil sample was placed on an extraction funnel equipped with a wire mesh and a 25-watt incandescent bulb. The bottom of the funnel contained 20 ml of 70% alcohol to collect mesofauna descending due to gradual drying over 48 hours. Mesofauna collected were transferred to Petri dishes and observed under a Zeiss microscope at 40× magnification for identification to the order level by two independent observers to ensure accuracy. Soil mesofauna population was calculated as individuals dm^{-1}. Soil mesofauna diversity index was calculated using the Shannon-Wiener index (Odum, 1983) (Equation 1).

$$H' = -\sum[(\frac{ni}{N}) \ln (\frac{ni}{N})] \quad (1)$$

The diversity index (H′) was calculated based on the proportion of the number of individuals of each species (ni) relative to the total number of individuals in the community (N). Soil mesofauna dominance index was calculated using Simpson's index (Krebs, 1989) (Equation 2). Where C represents the dominance index, n_i denotes the number of individuals of the *i*-th species, and *N* indicates the total number of individuals in the community.

$$C = \sum [(\frac{ni}{N})^2 \quad (2)$$

2.4.3. Soil Microbial Activity Analysis

Soil microbial activity was measured through soil respiration using the Verstraete method (Anas, 1989). 100 g of soil sample was placed in an incubation jar containing 5 ml of 0.2 N KOH to absorb CO_2 and 10 ml of distilled water to maintain humidity. The jar was sealed and incubated at room temperature in the dark for 7 days. After incubation, the KOH solution was titrated with 0.1 N HCl using phenolphthalein and methyl orange indicators. A blank without a soil sample was prepared as a control. Soil microbial activity was expressed as mg CO_2-C kg soil day^{-1} and calculated using Equation 3. Where *a* represents the volume of HCl used for sample titration, *b* denotes the volume of HCl used for blank titration, *t* indicates the normality of HCl, and *n* refers to the number of incubation days.

$$R = \frac{(a-b)\times t \times 120}{n} \quad (3)$$

2.4.4. Supporting Variables

Soil chemical properties were analyzed for organic-C (Walkley-Black), total-N (Kjeldahl), and pH (pH meter). Soil temperature was measured with a soil thermometer at a depth of 5-10 cm. Soil moisture was measured using a soil moisture tester.

2.5. Data Analysis

Data were analyzed using analysis of variance (ANOVA) at the 5% significance level in SAS 9.4. When significant effects were observed, Least Significant Difference (LSD) test at 5% was performed. The relationship between main variables and supporting variables was analyzed using Pearson correlation (n = 27).

3. Results and Discussion

3.1. Soil Properties

Soil chemical and physical properties per treatment during the sampling period are presented in Table 1. Soil pH ranged from 6.60 (B0P1) to 7.07 (B0P0), indicating near-neutral to slightly alkaline conditions across all treatments, which is more favorable than the strongly acidic conditions typically found in Ultisol soils. The organic-C content ranged from 1.42% (B0P0) to 1.95% (B0P1), while total-N ranged from 0.26% (B0P0) to 0.46% (B1P0), reflecting moderate soil fertility conditions typical of cultivated Ultisol. These moderate organic matter levels may have supported the mesofauna diversity observed in this study, as organic matter serves as the primary energy source and habitat for soil mesofauna communities (Menta and Remelli, 2020). Soil temperature varied from 27.08°C (B2P0) to 27.53°C (B2P2), and soil moisture ranged from 70.94% (B1P0) to 71.28% (B2P2),

indicating favorable and relatively uniform physical conditions for mesofauna activity across all treatments, corresponding with the transition to the rainy season in Bandar Lampung.

Table 1. Soil chemical and physical properties during the sampling period

Treatment	Organic-C (%)	Total-N (%)	pH	Soil temperature (°C)	Soil moisture (%)
B0P0	1.42	0.26	7.07	27.14	71.03
B0P1	1.95	0.33	6.60	27.27	71.09
B0P2	1.58	0.41	6.83	27.43	71.11
B1P0	1.51	0.46	6.97	27.28	70.94
B1P1	1.61	0.44	6.83	27.27	71.16
B1P2	1.65	0.36	6.87	27.39	71.25
B2P0	1.44	0.36	6.97	27.08	70.98
B2P1	1.51	0.30	6.90	27.33	71.19
B2P2	1.50	0.41	6.83	27.53	71.28

3.2. Soil Mesofauna Abundance by Order in Patchouli Cultivated Land

A total of 501 soil mesofauna individuals were identified across all treatments, comprising several orders: Collembola (145 individuals), Acarina (337 individuals), Symphyla (4 individuals), and unidentified taxa (15 individuals). The unidentified taxa refer to specimens whose morphological features were too damaged or incomplete for identification to the order level under microscopic observation. Orders Protura and Diplura were not found in any treatment. The dominant order across all treatments was Acarina, accounting for 67.3% of total mesofauna, followed by Collembola at 28.9%. The highest mesofauna abundance was observed in treatment B2P2 (second-year biochar residue at 20 ton ha^{-1} combined with NPK fertilizer at 700 kg ha^{-1}), with 84 individuals, while the lowest was in B0P0 (without biochar and without NPK), with 26 individuals (Figure 1). The higher abundance in B2P2 is likely attributed to increased organic inputs from NPK-stimulated plant growth, which enhanced root exudates and litter production as food resources for mesofauna communities, while the presence of aged biochar may have contributed to improved soil porosity and moisture retention as a physical microhabitat (Xiao et al., 2018; Palansooriya et al., 2019). Visual documentation of representative specimens from each identified order is presented in Figure 2, showing morphological characteristics that facilitated identification to order level.

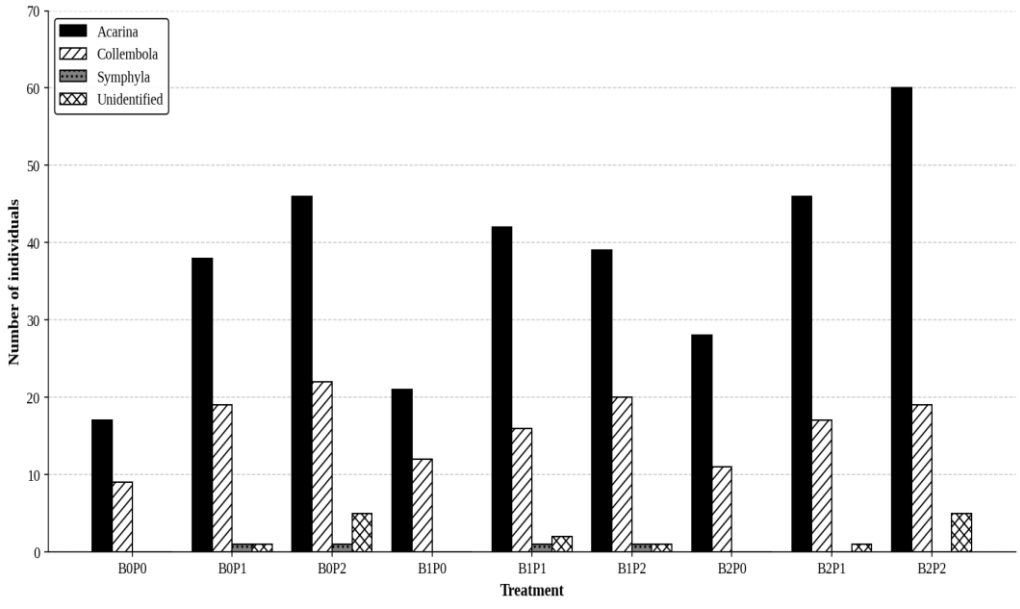

Figure 1. Soil mesofauna abundance by order across different treatments of second-year biochar residue and NPK fertilizer in patchouli cultivated land.

The dominance of Acarina aligns with previous findings indicating that mites are among the most abundant and widespread soil mesofauna in agricultural systems (Bedano et al., 2016). Acarina play crucial roles in decomposition processes, fungal regulation, and serve as predators of nematodes and other soil microarthropods,

functioning as both detritivores and predators of microfauna in soil food webs (Gao et al., 2020). The presence of Collembola as the second-most-abundant order reflects their ecological importance in fragmenting organic matter and facilitating microbial decomposition (Potapov et al., 2021).

Figure 2. Microscopic documentation of soil mesofauna specimens identified in this study: (A) Collembola; (B) Acarina; (C) Symphyla,; (D) Unidentified.

3.3. Soil Mesofauna Population

Analysis of variance revealed that second-year biochar residue treatments had no significant effect on mesofauna population (Table 2). However, NPK fertilizer application significantly affected mesofauna population ($p < 0.05$). The interaction between biochar residue and NPK fertilizer was not significant. The highest mesofauna population was observed in P2 treatment (NPK 700 kg ha^{-1}) at 24.33 ± 3.79 individuals dm^{-3}, significantly different from P1 (20.56 ± 2.42 individuals dm^{-3}) and P0 (10.89 ± 1.71 individuals dm^{-3}) (Table 3).

Table 2. Analysis of variance for soil mesofauna population, diversity index, dominance index, and microbial activity

Treatment	Variable			
	Mesofauna population	**Diversity index (H')**	**Dominance index (C)**	**Microbial activity**
Biochar (A)	ns	ns	ns	ns
NPK (B)	*	*	*	ns
A × B	ns	ns	ns	ns

Note: * = significant at 5% level; ns = not significant

Table 3. Mean effects of second-year biochar residue and NPK fertilizer on soil mesofauna population

	Treatment	**Mean (individuals dm^{-3})**
Second-year biochar residue	B0 (without biochar residue)	17.67 ± 6.11
	B1 (biochar residue 10 ton ha^{-1})	17.33 ± 5.74
	B2 (biochar residue 20 ton ha^{-1})	20.78 ± 6.42
NPK fertilizer	P0 (without NPK fertilizer)	10.89 ± 1.71 c
	P1(350 kg ha^{-1})	20.56 ± 2.42 b
	P2 (700 kg ha^{-1})	24.33 ± 3.79 a
	LSD 5%	2.70

Note: Means followed by different letters are significantly different at LSD 5% test

The lack of a significant effect from second-year biochar residue on mesofauna population can be attributed to biochar stabilization processes. This result contrasts with the study by Kusumastuti et al. (2022), who found significant biochar effects in the first year of application on the same patchouli plantation, with the best dose being 20 ton ha^{-1} combined with NPK fertilizer 700 kg ha^{-1}. This difference indicates that biochar effects on mesofauna communities are temporal and decline over time. After one to two years of application, biochar becomes integrated into the soil matrix and its direct influence on soil biota communities diminishes (Kuzyakov et al., 2009; Lehmann

et al., 2021). This stabilization reduces the availability of labile carbon, which initially stimulates mesofauna activity during the first year. The recalcitrant nature of biochar carbon means its effects on soil fauna communities become more indirect over time, primarily through long-term improvements in soil physical properties rather than as a direct food source (Palansooriya et al., 2019). Supporting soil physical data showed that soil temperature ranged from 27.08°C to 27.53°C and soil moisture ranged from 70.94% to 71.28% across all treatments during the sampling period, which corresponds with the transition to the rainy season. These physical conditions may have influenced mesofauna distribution patterns across treatments more strongly than biochar residue effects.

Conversely, NPK fertilization significantly enhanced mesofauna population through multiple mechanisms. The availability of nitrogen, phosphorus, and potassium promotes plant growth and root development, which in turn increases root exudates and fine-root turnover, thereby serving as energy sources for soil biota (Xiao et al., 2018). The increased plant biomass resulting from NPK fertilization provides greater organic inputs from leaf litter and root residues, creating favorable conditions for mesofauna communities (Zhu et al., 2020). Furthermore, NPK fertilization can indirectly enhance mesofauna populations by stimulating microbial communities that serve as food sources for bacterivores and fungivores such as Collembola and certain Acarina groups (Coulibaly et al., 2017).

3.4. Soil Mesofauna Diversity Index

The overall mean diversity index (H') was 0.24, indicating low diversity (H' < 1.5). Second-year biochar residue had no significant effect on the diversity index, while NPK fertilizer application significantly affected diversity (Table 2). Treatment P2 (NPK 700 kg ha^{-1}) showed the highest diversity index (0.28), significantly different from P1 (0.26) and P0 (0.18) (Table 4).

Table 4. Mean effects of second-year biochar residue and NPK fertilizer on soil mesofauna diversity index

	Treatment	Mean (H')
Second-year biochar residue	B0 (without biochar residue)	0.23 ± 0.06
	B1 (biochar residue 10 ton ha^{-1})	0.23 ± 0.04
	B2 (biochar residue 20 ton ha^{-1})	0.25 ± 0.04
NPK fertilizer	P0 (without NPK fertilizer)	0.18 ± 0.02 c
	P1(350 kg ha^{-1})	0.26 ± 0.02 b
	P2 (700 kg ha^{-1})	0.28 ± 0.01 b
	LSD 5%	0.019

Note: Means followed by different letters are significantly different at LSD 5% test

The low diversity index values across all treatments suggest environmental stress or limiting factors affecting mesofauna communities. Low diversity may result from extreme soil conditions such as pH imbalance, moisture stress, or temperature fluctuations during the sampling period (Menta and Remelli, 2020). The observed soil pH ranging from 6.60 to 7.07, organic-C content from 1.42% to 1.95%, and total-N from 0.26% to 0.46% across treatments reflect moderate fertility conditions in cultivated Ultisol, providing adequate habitat quality for mesofauna communities. The study was conducted during the transition to the rainy season, which may have caused environmental instability affecting mesofauna diversity. During the transition from the dry to the rainy season, increased soil moisture and fluctuating temperatures can disrupt mesofaunal community equilibrium, potentially favoring moisture-tolerant species, such as certain Acarina groups, and reducing the relative abundance of more sensitive taxa, thereby lowering overall diversity (Menta and Remelli, 2020).

The absence of effects of biochar residue on diversity aligns with findings that biochar's influence on soil fauna diversity decreases substantially after the first year due to surface oxidation and integration into soil aggregates (Lehmann et al., 2021). The initial colonization of fresh biochar surfaces by microorganisms and the subsequent attraction of mesofauna diminish as biochar ages and becomes less reactive (Palansooriya et al., 2019).

NPK fertilization enhanced mesofauna diversity by improving resource availability and niche differentiation. Higher nutrient availability promotes diverse plant and microbial communities, which in turn support varied mesofauna assemblages with different feeding strategies (Zhu et al., 2020). The significant increase in diversity with higher NPK rates suggests that nutrient enrichment can counteract environmental limitations and promote more complex soil food webs in patchouli cultivation systems.

3.5. Soil Mesofauna Dominance Index

Mean dominance index (C) across all treatments was 0.014, which is very low (C < 0.1), indicating low dominance and relatively balanced mesofauna community composition. Second-year biochar residue showed no significant

effect on the dominance index, while NPK fertilizer application significantly affected dominance (Table 2). Treatment P2 showed the highest dominance index (0.022), followed by P1 (0.015) and P0 (0.004) (Table 5).

Table 5. Mean effects of second-year biochar residue and NPK fertilizer on soil mesofauna dominance index

	Treatment	Mean (C)
Second-year biochar residue	B0 (without biochar residue)	0.013 ± 0.009
	B1 (biochar residue 10 ton ha^{-1})	0.012 ± 0.007
	B2 (biochar residue 20 ton ha^{-1})	0.017 ± 0.009
NPK fertilizer	P0 (without NPK fertilizer)	0.004 ± 0.002 c
	P1(350 kg ha^{-1})	0.015 ± 0.004 b
	P2 (700 kg ha^{-1})	0.022 ± 0.005 a
	LSD 5%	0.004

Note: Means followed by different letters are significantly different at LSD 5% test

Dominance index values below 0.1 indicate a balanced community structure in which no single taxon overwhelmingly dominates the mesofaunal assemblage (Magurran, 2004). Although Acarina was numerically dominant in absolute abundance, functioning primarily as detritivores and predators of microfauna, the very low dominance index suggests the presence of multiple coexisting taxa contributing to community structure.

The lack of an effect of biochar residue on dominance patterns may be explained by the uniform influence of aged biochar on soil microhabitat conditions. After integration into the soil matrix, biochar provides relatively homogeneous microsites that do not preferentially favor specific mesofauna taxa (Dai et al., 2021). The recalcitrant nature of second-year biochar residue limits its ability to create distinct ecological niches that would promote dominance shifts.

NPK fertilization increased dominance index values, though remaining very low, suggesting slight shifts toward more dominant taxa. This pattern may reflect competitive advantages gained by certain mesofauna groups under nutrient-enriched conditions. Higher nutrient availability can favor fast-growing, opportunistic species that respond rapidly to resource pulses, potentially increasing their relative abundance (Gao et al., 2020). However, the overall very low dominance values indicate that NPK fertilization did not severely disrupt community balance, maintaining ecological stability in the soil system.

3.6. Soil Microbial Activity

Soil microbial activity showed no significant response to either second-year biochar residue or NPK fertilizer application, nor their interaction (Table 2). Microbial activity ranged from 0.57 to 3.54 mg CO_2-C kg soil day^{-1}, with considerable variation among treatments (Table 6). The coefficient of variation for microbial activity was 36.5%, indicating substantial spatial and temporal heterogeneity in microbial respiration rates across experimental units.

Table 6. Mean soil microbial activity across second-year biochar residue and NPK fertilizer treatments

	Treatment	Mean (mg CO_2-C kg soil day^{-1})
Second-year biochar residue	B0 (without biochar residue)	1.31
	B1 (biochar residue 10 ton ha^{-1})	0.76
	B2 (biochar residue 20 ton ha^{-1})	2.30
NPK fertilizer	P0 (without NPK fertilizer)	0.88
	P1(350 kg ha^{-1})	1.70
	P2 (700 kg ha^{-1})	1.81
	CV (%)	36.5

The lack of significant effects on microbial activity contrasts with the responses of mesofauna, suggesting different temporal dynamics between microbial and faunal communities. Soil microbial activity is highly sensitive to short-term environmental fluctuations and substrate availability, potentially masking treatment effects under field conditions (Trivedi et al., 2016).

The absence of effects of biochar residue on microbial activity aligns with findings that aged biochar contributes minimal labile carbon to microbial metabolism compared to fresh biochar (Wang et al., 2016). After approximately two years of application, most easily decomposable carbon fractions in biochar have been consumed or transformed, leaving predominantly recalcitrant aromatic structures that are not readily metabolized

by soil microorganisms (Lehmann et al., 2021). This result contrasts with findings by Kusumastuti et al. (2022) in the first year, showing higher microbial activity under biochar treatments, confirming that the stimulatory effect of biochar on microbial activity is temporary and decreases in the second year. To maintain long-term soil microbial activity, periodic organic amendment applications, such as compost or green manure, alongside biochar may be necessary to sustain labile carbon pools that drive microbial metabolism (Agegnehu et al., 2016).

Similarly, the lack of an effect of NPK fertilizer on microbial activity may indicate that microbial communities in this system are not primarily limited by inorganic nutrients but rather by organic carbon availability or environmental factors such as moisture and temperature (Sun et al., 2019). Microbial respiration is driven primarily by labile organic carbon substrates. While NPK fertilization can indirectly affect microbial activity through increased plant inputs, this effect may not be immediately detectable in short-term measurements (Trivedi et al., 2016).

3.7. Correlation Main Variables and Supporting Variables

Pearson correlation analysis (n = 27) revealed significant positive relationships between mesofauna variables and environmental factors (Table 7). Mesofauna population showed strong positive correlations with soil temperature ($r = 0.74$, $p < 0.05$) and soil moisture ($r = 0.83$, $p < 0.05$). Similarly, the diversity index was significantly correlated with soil temperature ($r = 0.70$, $p < 0.05$) and soil moisture ($r = 0.81$, $p < 0.05$). The dominance index showed a significant positive correlation with soil temperature ($r = 0.78$, $p < 0.05$) but not with soil moisture. No significant correlations were found between mesofauna variables and soil chemical properties (organic-C, total-N, pH) or between microbial activity and any supporting variables.

Table 7. Correlation coefficients between main variables and supporting variables

Variable	R Value			
	Mesofauna Population	**Diversity Index**	**Dominance Index**	**Microbial Activity**
Total-N	0.28 ns	0.28 ns	0.27 ns	0.37 ns
Organic-C	0.31 ns	0.37 ns	0.23 ns	-0.22 ns
pH	-0.61 ns	-0.66 ns	-0.54 ns	0.01 ns
Soil Temperature	0.74 *	0.70 *	0.78 *	0.44 ns
Soil moisture	0.83 *	0.81 *	0.38 ns	0.59 ns

Note: * = significant; ns = not significant

Correlations between mesofauna variables and soil physical conditions (temperature and moisture) highlight the primary importance of microclimate in determining mesofauna distribution and activity. Soil temperature directly affects mesofauna metabolic rates, locomotion, and reproductive cycles (Menta and Remelli, 2020). Soil temperature across treatments ranged from 27.08°C to 27.53°C, which falls within the optimal range for mesofauna activity in tropical agricultural soils, facilitating increased activity, foraging efficiency, and population growth. The positive correlation ($r = 0.74$) suggests that temperature variation among plots was a significant driver of differences in mesofauna populations.

Soil moisture showed the strongest correlation with mesofauna population and diversity, emphasizing the critical role of water availability for soil mesofauna. Soil moisture across treatments ranged from 70.94% to 71.28%, indicating favorable water availability, which is essential for survival and activity (Potapov et al., 2021). Adequate soil moisture also facilitates mesofauna movement through soil pores and supports the microbial food sources upon which many mesofauna depend. The high correlation coefficient ($r = 0.83$) indicates that moisture was a primary driver of mesofauna population patterns in this study, potentially overshadowing treatment effects.

The absence of significant correlations between mesofauna variables and soil chemical properties (organic-C, total N, pH) suggests that within the range of values observed in this study (organic-C = 1.42-1.95%, total-N = 0.26-0.46%, pH = 6.60-7.07), these factors were not limiting for mesofauna communities. This finding contrasts with the significant treatment effects of NPK fertilization, suggesting that fertilizer effects on mesofauna operate primarily through indirect pathways, such as enhanced plant growth and organic matter inputs, rather than through direct responses to soil nutrient concentrations (Zhu et al., 2020).

4. CONCLUSION

Second-year biochar residue showed no significant effects on soil mesofauna abundance, diversity index, dominance index, or soil microbial activity in patchouli cultivation. This finding indicates that the stimulatory effects of biochar on soil biota communities diminish substantially after the first year due to stabilization and

integration into the soil matrix, thereby reducing labile carbon availability, which initially promotes biological activity. In contrast, NPK fertilizer application significantly influenced soil mesofauna communities, with the highest rate (700 kg ha^{-1}) resulting in the greatest mesofauna population (24.33 individuals dm^{-3}), highest diversity index (0.28), and highest dominance index (0.022), demonstrating its role in enhancing soil biological health as an indicator of cultivation system sustainability. However, NPK fertilization did not significantly affect soil microbial activity. Soil physical factors, particularly temperature (r = 0.74) and moisture (r = 0.83), showed stronger correlations with mesofauna abundance than soil chemical properties, including organic-C (1.42-1.95%), total-N (0.26-0.46%), and pH (6.60-7.07). No significant interactions were observed between second-year biochar residue and NPK fertilizer for any measured variables.

References

Agegnehu G, Bass, A.M., Nelson, P.N., Bird, M.I. 2016. Benefits of biochar, compost and biochar-compost for soil quality, maize yield and greenhouse gas emissions in a tropical agricultural soil. Science of the Total Environment 543, 295-306. https://doi.org/10.1016/j.scitotenv.2015.11.054

Anas, I. 1989. Biologi Tanah dalam Praktek. Institut Pertanian Bogor, Bogor. (in Indonesian)

Bedano, J.C., Domínguez, A., Arolfo, R., Wall, D.H. 2016. Effect of good agricultural practices under no-till on litter and soil invertebrates in areas with different soil types. Soil and Tillage Research 158, 100-109. https://doi.org/10.1016/j.still.2015.12.005

Blanco-Canqui, H. 2017. Biochar and soil physical properties. Soil Science Society of America Journal 81(4), 687-711. https://doi.org/10.2136/sssaj2017.01.0017

Coulibaly, S.F.M., Winck, B.R., Akpa-Vinceslas, M., Mignot, L., Legras, M., Forey, E., Chauvat, M. 2017. Functional assemblages of Collembola determine soil microbial communities and associated functions. Frontiers in Environmental Science 5, 81. https://doi.org/10.3389/fenvs.2017.00081

Dai, Z., Zhang, X., Tang, C., Muhammad, N., Wu, J., Brookes, P.C., Xu, J. 2021. Potential role of biochars in decreasing soil acidification -A critical review. Science of the Total Environment 753, 142043. https://doi.org/10.1016/j.scitotenv.2020.142043

Gao, M., Taylor, M.K., Callaham Jr, M.A. 2020. Trophic ecology of soil mesofauna in a successional gradient of montane forests. Soil Biology and Biochemistry 148, 107867. https://doi.org/10.1016/j.soilbio.2020.107867

Kementerian Pertanian. 2024. Outlook Komoditas Perkebunan Nilam. Pusat Data dan Sistem Informasi Pertanian, Jakarta. (in Indonesian)

Krebs, C.J. 1989. Ecological Methodology. Harper and Row Publishers, New York.

Kusumastuti, A., Indrawat,i W., Kurniawan, A. 2022. Keanekaragaman mesofauna tanah dan aktivitas mikroorganisme tanah pada vegetasi nilam di berbagai dosis biochar dan pupuk majemuk NPK. Agriprima 6(2), 145-162. https://doi.org/10.25047/agriprima.v6i2.488 (in Indonesian)

Kuzyakov, Y., Subbotina, I., Chen, H., Bogomolova, I., Xu, X. 2009. Black carbon decomposition and incorporation into soil microbial biomass estimated by ^{14}C labeling. Soil Biology and Biochemistry 41(2), 210-219. https://doi.org/10.1016/j.soilbio.2008.10.016

Lehmann, J., Cowie, A., Masiello, C.A., Kammann, C., Woolf, D., Amonette, J.E., Cayuela, M.L., Camps-Arbestain, M., Whitman, T. 2021. Biochar in climate change mitigation. Nature Geoscience 14(12), 883-892. https://doi.org/10.1038/s41561-021-00852-8

Magurran, A.E. 2004. Measuring Biological Diversity. Blackwell Publishing, Oxford.

Menta, C., Remelli, S. 2020. Soil health and arthropods: From complex system to worthwhile investigation. Insects 11(1), 54. https://doi.org/10.3390/insects11010054

Nisa, K., Sufardi, S., Rusdi, M., Indra, I. 2024. Soil quality index and patchouli yields on various cropping systems in Aceh province, Indonesia: Case study in Aceh Barat Regency. Case Studies in Chemical and Environmental Engineering 10, 100798. https://doi.org/10.1016/j.cscee.2024.100798

Odum, E.P. 1983. Basic Ecology. Saunders College Publishing, Philadelphia.

Palansooriya, K.N., Ok, Y.S., Awad, Y.M., Lee, S.S., Sung, J.K., Koutsospyros, A., Moon, D.H. 2019. Impacts of biochar application on upland agriculture: A review. Journal of Environmental Management 234, 52-64. https://doi.org/10.1016/j.jenvman.2018.12.085

Potapov, A.M., Tiunov, A.V., Scheu, S. 2021. Uncovering trophic positions and food resources of soil animals using bulk natural stable isotope composition. Biological Reviews 94(1), 37-59. https://doi.org/10.1111/brv.12434

Sun, H., Lu, H., Chu, L., Shao, H., Shi, W. 2019. Biochar applied with appropriate rates can reduce N leaching, keep N retention and not increase NH_3 volatilization in a coastal saline soil. Science of the Total Environment 575, 820-825. https://doi.org/10.1016/j.scitotenv.2016.09.137

Trivedi, P., Delgado-Baquerizo, M., Trivedi, C., Hu, H., Anderson, I.C., Jeffries, T.C., Zhou, J., Singh, B.K. 2016. Microbial regulation of the soil carbon cycle: Evidence from gene-enzyme relationships. The ISME Journal 10, 2593-2604. https://doi.org/10.1038/ismej.2016.65

Wang, J., Xiong, Z., Kuzyakov, Y. 2016. Biochar stability in soil: Meta-analysis of decomposition and priming effects. GCB Bioenergy 8(3), 512-523. https://doi.org/10.1111/gcbb.12266

Wang, X., Zeeshan Ul Haq, M., Yu, J., Liu, Y., Yang, H., Cui, H., Yang, D., Wu, Y. 2024. Continuous cropping of patchouli alleviate soil properties, enzyme activities, and bacterial community structures. Plants 13(24), 3481. https://doi.org/10.3390/plants13243481

Xiao, L., Yuan, G., Feng, L., Bi, D., Wei, J. 2018. Soil properties and the growth of wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) in response to reed (*Phragmites communis*) biochar use in a salt-affected soil in the Yellow River Delta. Agriculture, Ecosystems & Environment 258, 51-60. https://doi.org/10.1016/j.agee.2018.02.010

Zhu, Z., Bai, Y., Lv, M., Tian, G., Zhang, X., Li, L., Jiang, Y., Ge, S. 2020. Soil fertility, microbial biomass, and microbial functional diversity responses to four years fertilization in an apple orchard in North China. Horticultural Plant Journal 6(4), 223-230. https://doi.org/10.1016/j.hpj.2020.06.002